Rhesus Monkey CEA Codon-Optimized Nucleotide Sequence

```
   1  ATGGGCAGCC CCAGCGCCCC CCTGCACCGC TGGTGCATCC CCTGGCAGAC
      CCTGCTGCTG ACCGCCAGCC TGCTGACCTT CTGGAACCCC CCCACCACCG
 101  CCCAGCTGAC CATCGAGAGC CGCCCCTTCA ACGTGGCCGA GGGCAAGGAG
      GTGCTGCTGC TGGCCCACAA CGTGAGCCAG AACCTGTTCG GCTACATCTG
 201  GTACAAGGGC GAGCGCGTGG ACGCCAGCCG CCGCATCGGC AGCTGCGTGA
      TCCGCACCCA GCAGATCACC CCCGGCCCCG CCCACAGCGG CCGCGAGACC
 301  ATCGACTTCA ACGCCAGCCT GCTGATCCAC AACGTGACCC AGAGCGACAC
      CGGCAGCTAC ACCATCCAGG TGATCAAGGA GGACCTGGTG AACGAGGAGG
 401  CCACCGGCCA GTTCCGCGTG TACCCCGAGC TGCCCAAGCC CTACATCAGC
      AGCAACAACA GCAACCCCGT GGAGGACAAG GACGCCGTGG CCCTGACCTG
 501  CGAGCCCGAG ACCCAGGACA CCACCTACCT GTGGTGGGTG AACAACCAGA
      GCCTGCCCGT GAGCCCCCGC CTGGAGCTGA GCAGCGACAA CCGCACCCTG
 601  ACCGTGTTCA ACATCCCCCG CAACGACACC ACCAGCTACA AGTGCGAGAC
      CCAGAACCCC GTGAGCGTGC GCCGCAGCGA CCCCGTGACC CTGAACGTGC
 701  TGTACGGCCC CGACGCCCCC ACCATCAGCC CCCTGAACAC CCCCTACCGC
      GCCGGCGAGA ACCTGAACCT GACCTGCCAC GCCGCCAGCA ACCCCACCGC
 801  CCAGTACTTC TGGTTCGTGA ACGGCACCTT CCAGCAGAGC ACCCAGGAGC
      TGTTCATCCC CAACATCACC GTGAACAACA GCGGCAGCTA CATGTGCCAG
 901  GCCCACAACA GCGCCACCGG CCTGAACCGC ACCACCGTGA CCGCCATCAC
      CGTGTACGCC GAGCTGCCCA AGCCCTACAT CACCAGCAAC AACAGCAACC
1001  CCATCGAGGA CAAGGACGCC GTGACCCTGA CCTGCGAGCC CGAGACCCAG
      GACACCACCT ACCTGTGGTG GGTGAACAAC CAGAGCCTGA GCGTGAGCAG
1101  CCGCCTGGAG CTGAGCAACG ACAACCGCAC CCTGACCGTG TTCAACATCC
      CCCGCAACGA CACCACCTTC TACGAGTGCG AGACCCAGAA CCCCGTGAGC
1201  GTGCGCCGCA GCGACCCCGT GACCCTGAAC GTGCTGTACG GCCCCGACGC
      CCCCACCATC AGCCCCCTGA ACACCCCCTA CCGCGCCGGC GAGAACCTGA
1301  ACCTGAGCTG CCACGCCGCC AGCAACCCCG CCGCCCAGTA CAGCTGGTTC
      GTGAACGGCA CCTTCCAGCA GAGCACCCAG GAGCTGTTCA TCCCCAACAT
1401  CACCGTGAAC AACAGCGGCA GCTACATGTG CCAGGCCCAC AACAGCGCCA
      CCGGCCTGAA CCGCACCACC GTGACCGCCA TCACCGTGTA CGTGGAGCTG
1501  CCCAAGCCCT ACATCAGCAG CAACAACAGC AACCCCATCG AGGACAAGGA
      CGCCGTGACC CTGACCTGCG AGCCCGTGGC CGAGAACACC ACCTACCTGT
1601  GGTGGGTGAA CAACCAGAGC CTGAGCGTGA GCCCCCGCCT GCAGCTGAGC
      AACGGCAACC GCATCCTGAC CCTGCTGAGC GTGACCCGCA ACGACACCGG
1701  CCCCTACGAG TGCGGCATCC AGAACAGCGA GAGCGCCAAG CGCAGCGACC
      CCGTGACCCT GAACGTGACC TACGGCCCCG ACACCCCCAT CATCAGCCCC
1801  CCCGACCTGA GCTACCGCAG CGGCGCCAAC CTGAACCTGA GCTGCCACAG
      CGACAGCAAC CCCAGCCCCC AGTACAGCTG GCTGATCAAC GGCACCCTGC
1901  GCCAGCACAC CCAGGTGCTG TTCATCAGCA AGATCACCAG CAACAACAGC
      GGCGCCTACG CCTGCTTCGT GAGCAACCTG GCCACCGGCC GCAACAACAG
2001  CATCGTGAAG AACATCAGCG TGAGCAGCGG CGACAGCGCC CCCGGCAGCA
      GCGGCCTGAG CGCCCGCGCC ACCGTGGGCA TCATCATCGG CATGCTGGTG
2101  GGCGTGGCCC TGATGTGA (SEQ ID NO:1)
```

FIG.1A

Predicted Amino Acid Sequences of Rhesus Monkey CEA Proteins

```
  1   MGSPSAPLHR WCIPWQTLLL TASLLTFWNP PTTAQLTIES RPFNVAEGKE
 51   VLLLAHNVSQ NLFGYIWYKG ERVDASRRIG SCVIRTQQIT PGPAHSGRET
101   IDFNASLLIH NVTQSDTGSY TIQVIKEDLV NEEATGQFRV YPELPKPYIS
151   SNNSNPVEDK DAVALTCEPE TQDTTYLWWV NNQSLPVSPR LELSSDNRTL
201   TVFNIPRNDT TSYKCETQNP VSVRRSDPVT LNVLYGPDAP TISPLNTPYR
251   AGENLNLTCH AASNPTAQYF WFVNGTFQQS TQELFIPNIT VNNSGSYMCQ
301   AHNSATGLNR TTVTAITVYA ELPKPYITSN NSNPIEDKDA VTLTCEPETQ
351   DTTYLWWVNN QSLSVSSRLE LSNDNRTLTV FNIPRNDTTF YECETQNPVS
401   VRRSDPVTLN VLYGPDAPTI SPLNTPYRAG ENLNLSCHAA SNPAAQYSWF
451   VNGTFQQSTQ ELFIPNITVN NSGSYMCQAH NSATGLNRTT VTAITVYVEL
501   PKPYISSNNS NPIEDKDAVT LTCEPVAENT TYLWWVNNQS LSVSPRLQLS
551   NGNRILTLLS VTRNDTGPYE CGIQNSESAK RSDPVTLNVT YGPDTPIISP
601   PDLSYRSGAN LNLSCHSDSN PSPQYSWLIN GTLRQHTQVL FISKITSNNS
651   GAYACFVSNL ATGRNNSIVK NISVSSGDSA PGSSGLSARA TVGIIIGMLV
701   GVALM (SEQ ID NO:2)


  1   MGSPSAPLHR WCIPWQTLLL TASLLTFWNP PTTAQLTIES RPFNVAEGKE
 51   VLLLAHNVSQ NLFGYIWYKG ERVDASRRIG SCVIRTQQIT PGPAHSGRET
101   IDFNASLLIH NVTQSDTGSY TIQVIKEDLV NEEATGQFRV YPELPKPYIS
151   SNNSNPVEDK DAVALTCEPE TQDTTYLWWV NNQSLPVSPR LELSSDNRTL
201   TVFNIPRNDT TSYKCETQNP VSVRRSDPVT LNVLYGPDAP TISPLNTPYR
251   AGENLNLTCH AASNPTAQYF WFVNGTFQQS TQELFIPNIT VNNSGSYMCQ
301   AHNSATGLNR TTVTAITVYA ELPKPYITSN NSNPIEDKDA VTLTCEPETQ
351   DTTYLWWVNN QSLSVSSRLE LSNDNRTLTV FNIPRNDTTF YECETQNPVS
401   VRRSDPVTLN VLYGPDAPTI SPLNTPYRAG ENLNLSCHAA SNPAAQYFWF
451   VNGTFQQSTQ ELFIPNITVN NSGSYMCQAH NSATGLNRTT VTAITVYVEL
501   PKPYISSNNS NPIEDKDAVT LTCEPVAENT TYLWWVNNQS LSVSPRLQLS
551   NGNRILTLLS VTRNDTGPYE CGIQNSESAK RSDPVTLNVT YGPDTPIISP
601   PDLSYRSGAN LNLSCHSDSN PSPQYSWLIN GTLRQHTQVL FISKITSNNN
651   GAYACFVSNL ATGRNNSIVK NISVSSGDSA PGSSGLSARA TVGIIIGMLV
701   GVALM (SEQ ID NO:3)
```

FIG.1B

In Vitro Expression of rhCEA and rhCEAopt.

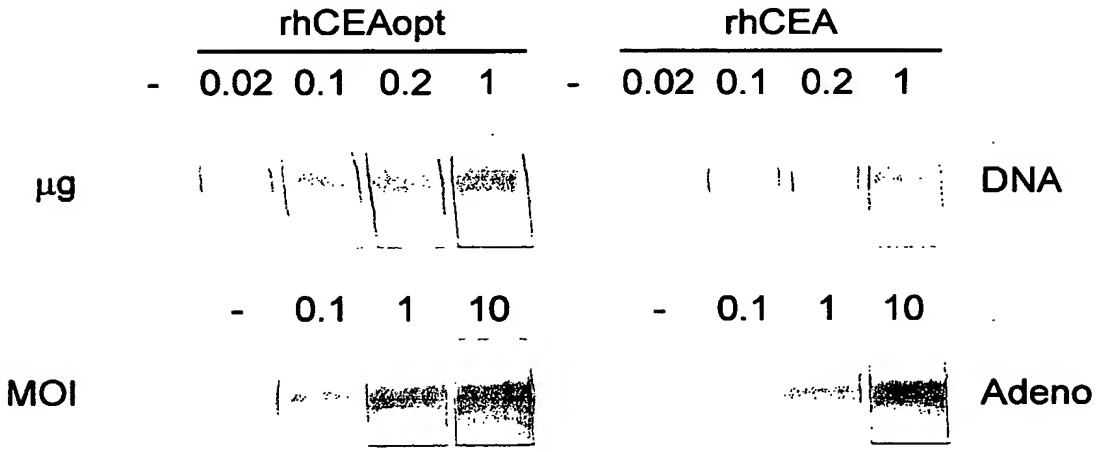

FIG.2

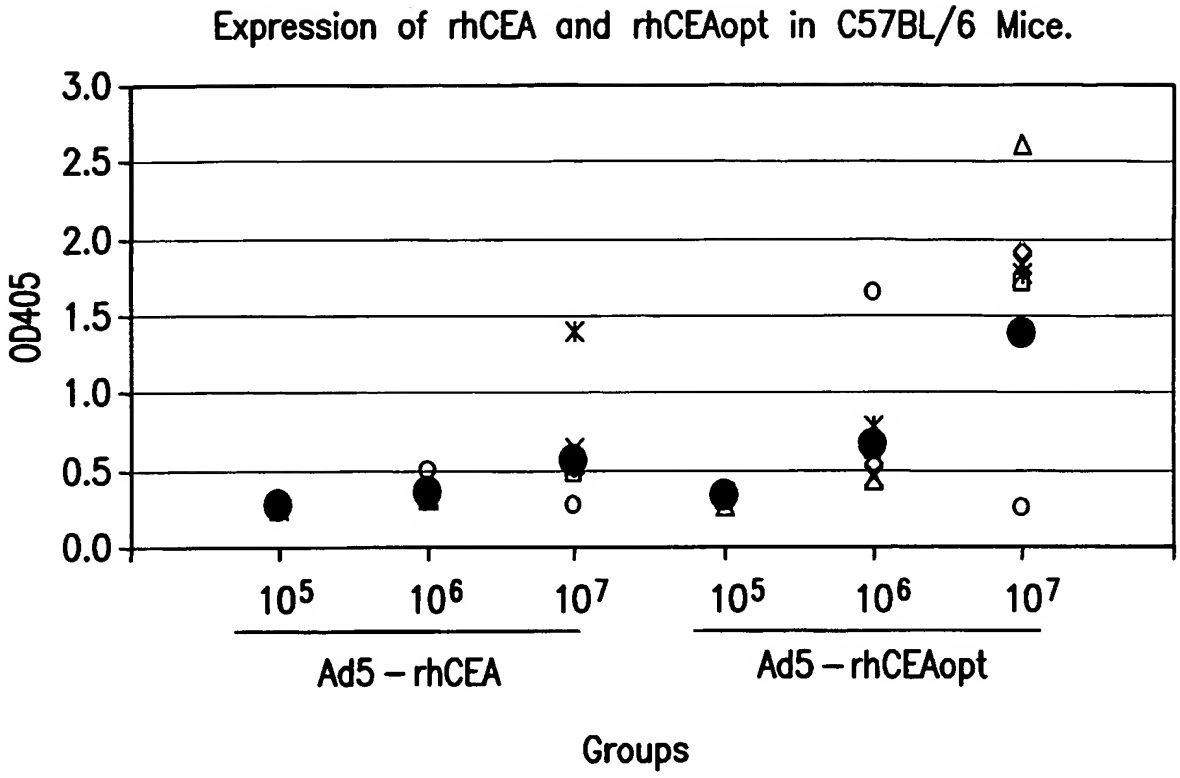
Expression of rhCEA and rhCEAopt in C57BL/6 Mice.
OD405
3.0
2.5
2.0
1.5
1.0
0.5
0.0
10^5
10^6
10^7
10^5
10^6
10^7
Ad5 – rhCEA
Ad5 – rhCEAopt
Groups

FIG.3

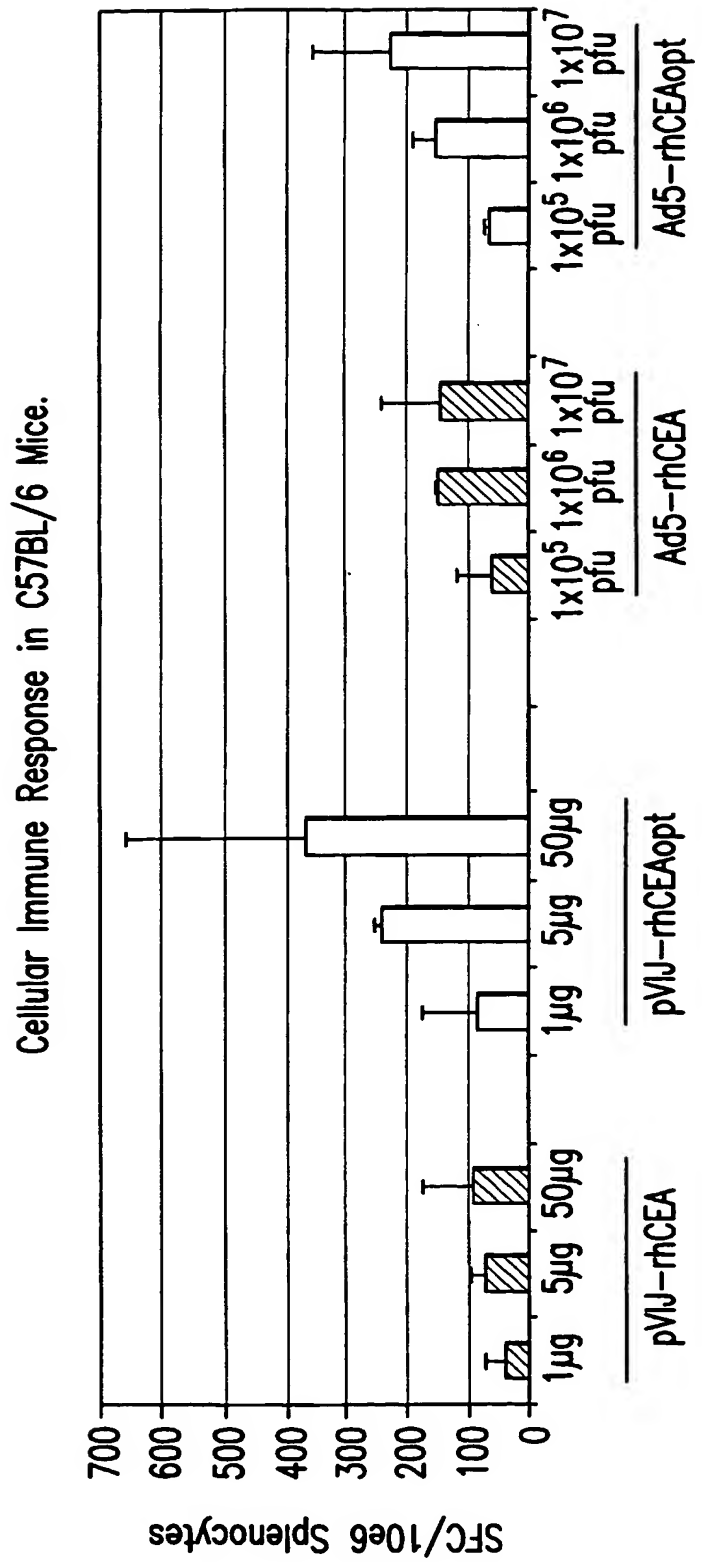
Cellular Immune Response in C57BL/6 Mice.
SFC/10e6 Splenocytes
700
600
500
400
300
200
100
0
1µg 5µg 50µg 1µg 5µg 50µg 1x10^5 pfu 1x10^6 pfu 1x10^7 pfu 1x10^5 pfu 1x10^6 pfu 1x10^7 pfu
pVIJ-rhCEA
pVIJ-rhCEAopt
Ad5-rhCEA
Ad5-rhCEAopt
Immunized Group

FIG.4

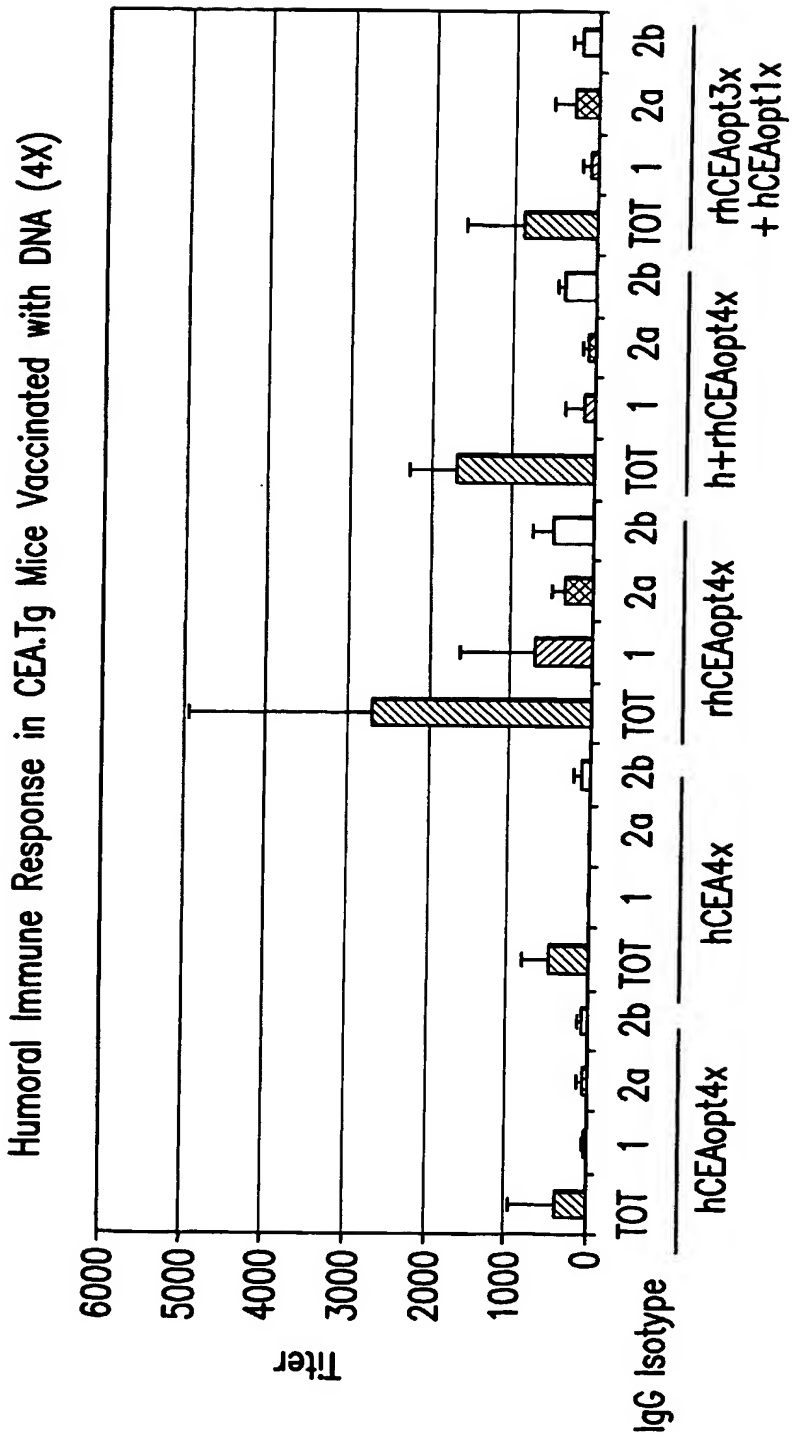
Humoral Immune Response in CEA.Tg Mice Vaccinated with DNA (4X)
Titer
6000
5000
4000
3000
2000
1000
0
IgG Isotype
TOT 1 2a 2b TOT 1 2a 2b TOT 1 2a 2b TOT 1 2a 2b TOT 1 2a 2b
hCEAopt4x
hCEA4x
rhCEAopt4x
h+rhCEAopt4x
rhCEAopt3x + hCEAopt1x

FIG.5

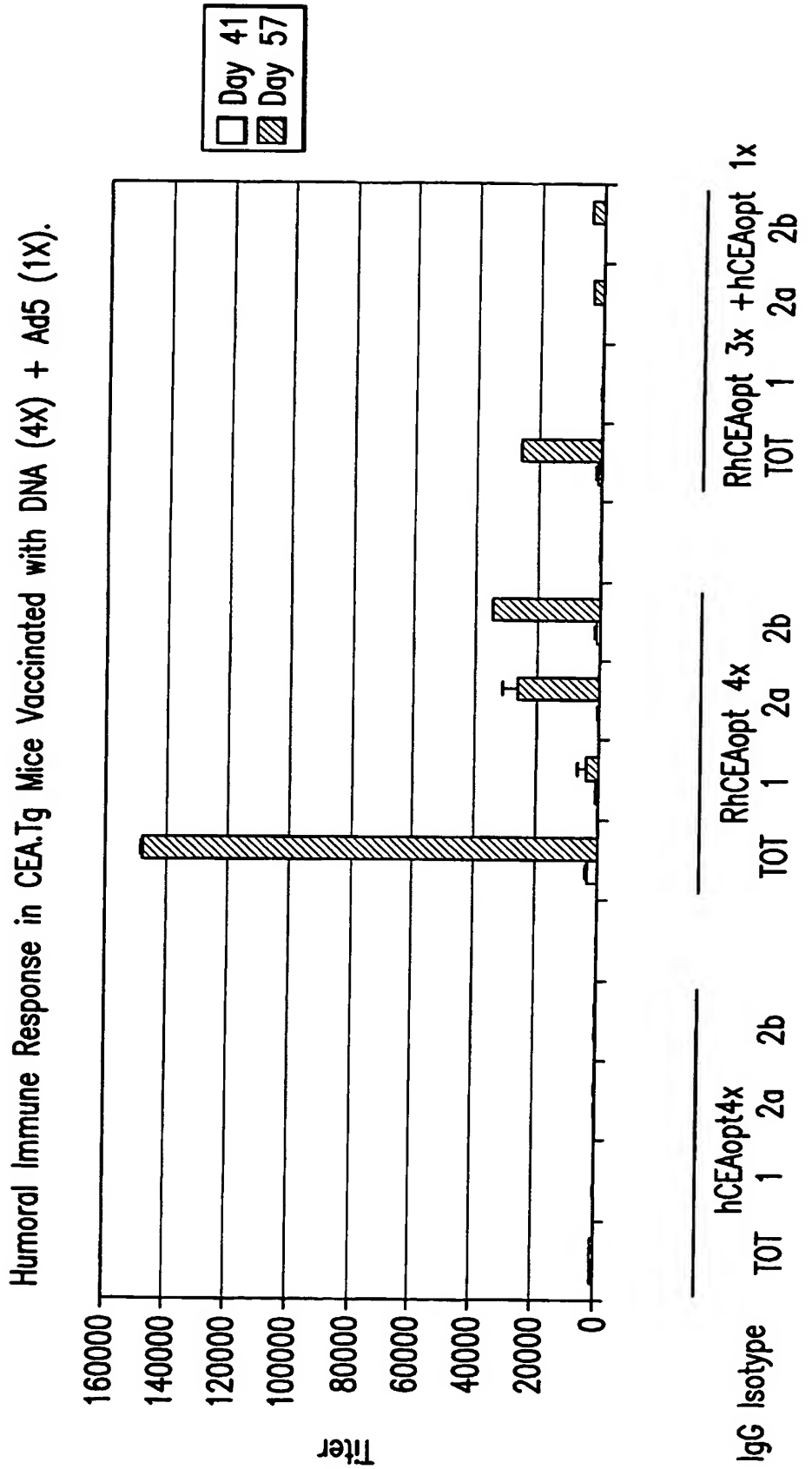
Humoral Immune Response in CEA.Tg Mice Vaccinated with DNA (4X) + Ad5 (1X).
Day 41
Day 57
Titer
160000
140000
120000
100000
80000
60000
40000
20000
0
IgG Isotype
TOT
1
2a
2b
hCEAopt4x
RhCEAopt 4x
RhCEAopt 3x +hCEAopt 1x

FIG.6

Cellular Immune Response in CEA.Tg Mice Immunized by DNA–Ad5 Mixed Modality

	IFNγ SFC/10^6					
	A	B	C	D	CD8+	DMSO
hCEA	0	2	3	9	1	0
hCEAopt	44	3	5	459	503	2
RhCEA	2	19	18	17	4	0
RhCEAopt	13	51	36	58	5	2
Rh/hCEAopt mix	16	12	17	190	257	6
Rh3x–hum1x	83	79	56	359	201	4

FIG.7

Cellular Immune Response of CEA.Tg Mice Immunized with Rhesus CEA Epitopes.

SFC/10e6	hCEAopt 4x + Ad5-hCEAopt ELISPOT Human	ELISPOT Rhesus	RhCEAopt 4x + Ad5-RhCEAopt ELISPOT Human	ELISPOT Rhesus	RhCEAopt 3x, hCEAopt 1x + Ad5-hCEAopt ELISPOT Human	ELISPOT Rhesus
CEA-5		8 ND	10	8	44	4
CEA-22		0 ND	8	32	0	18
CEA-35		2 ND	20	20	30	20
CEA-44		2 ND	38	12	14	10
CEA-45		0 ND	66	60	28	64
CEA-58		2 ND	0	0	2	16
CEA-65		4 ND	4	0	20	4
CEA-76		0 ND	8	16	4	12
CEA-77		6 ND	10	28	6	4
CEA-82		6 ND	0	4	10	2
CEA-88		4 ND	6	8	4	10
CEA-89		0 ND	54	12	64	8
CEA-90		4 ND	16	0	4	4
CEA-99		6 ND	4	24	6	2
CEA-100		6 ND	4	16	2	0
CEA-109		0 ND	14	4	8	2
CEA-110		2 ND	10	200	22	74
CEA-114		2 ND	6	0	10	4
CEA-121		4 ND	4	92	6	4
CEA-124		2 ND	6	16	4	12
CEA-131		0 ND	4	348	10	128
CEA-134		0 ND	0	26	4	16
CEA-142		2 ND	12	56	4	12
CEA-143	ND	ND	ND	ND	ND	16
CEA-163		0 ND	32	20	28	16
CEA-172		4 ND	4	0	20	0
DMSO	1 to 4		0		2	

CD8+Epitope

CD4+Epitope

CD4+/CD8+Epitope

FIG.8

In Vitro Expression of Ad24-rhCEAopt and Ad5-rhCEAopt

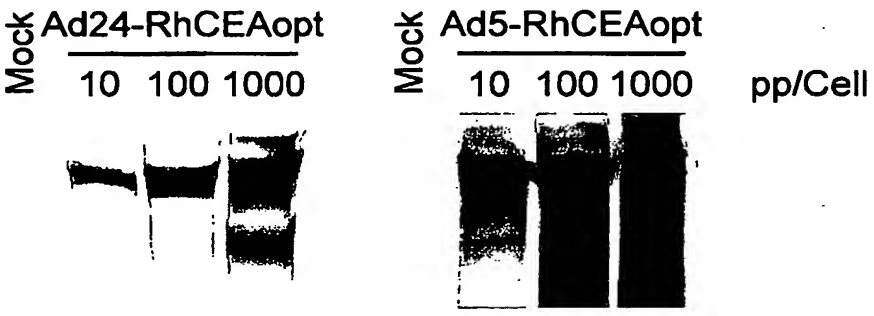

FIG.9

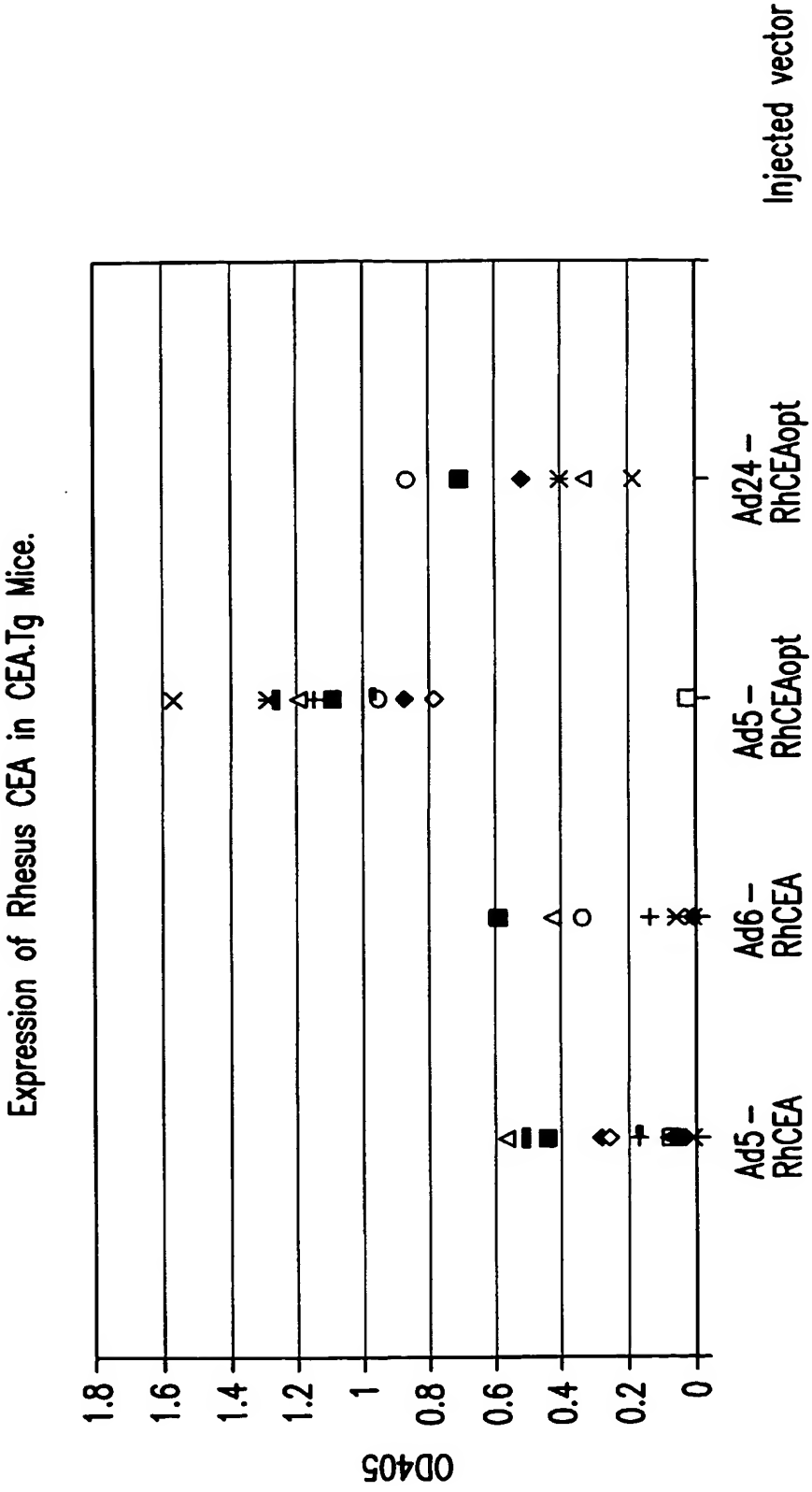
Expression of Rhesus CEA in CEA.Tg Mice.
OD405
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Ad5 – RhCEA
Ad6 – RhCEA
Ad5 – RhCEAopt
Ad24 – RhCEAopt
Injected vector

FIG. 10

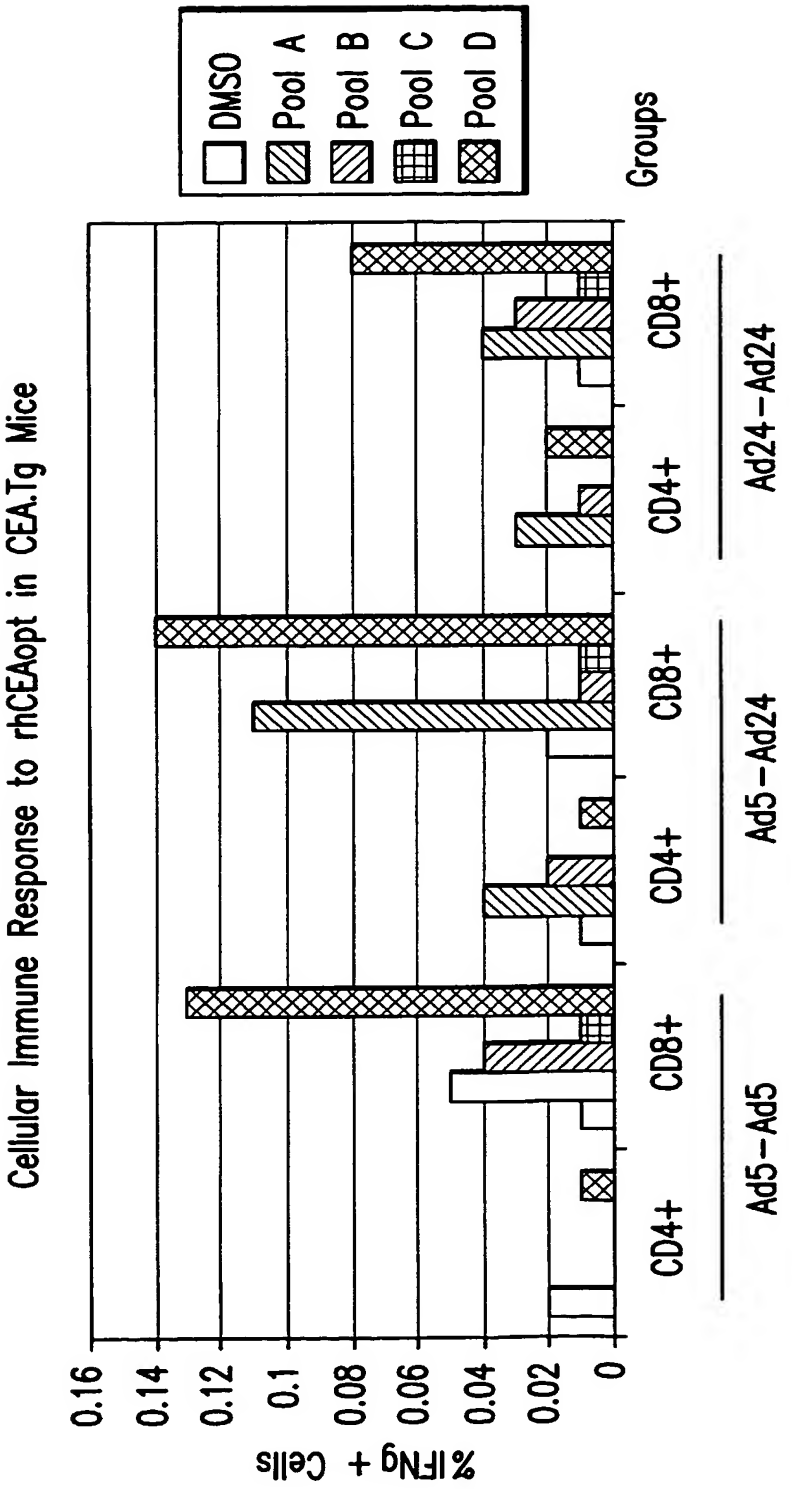
Cellular Immune Response to rhCEAopt in CEA.Tg Mice
% IFNg + Cells
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
DMSO
Pool A
Pool B
Pool C
Pool D
Groups
CD4+
CD8+
CD4+
CD8+
CD4+
CD8+
Ad5-Ad5
Ad5-Ad24
Ad24-Ad24

FIG.11

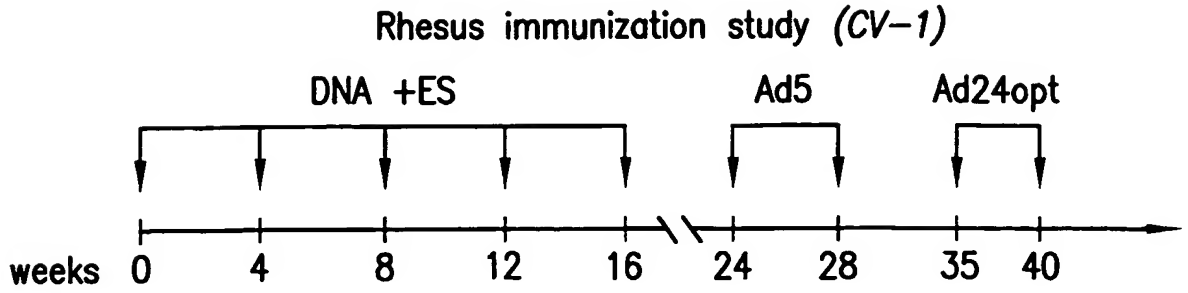

FIG.12A

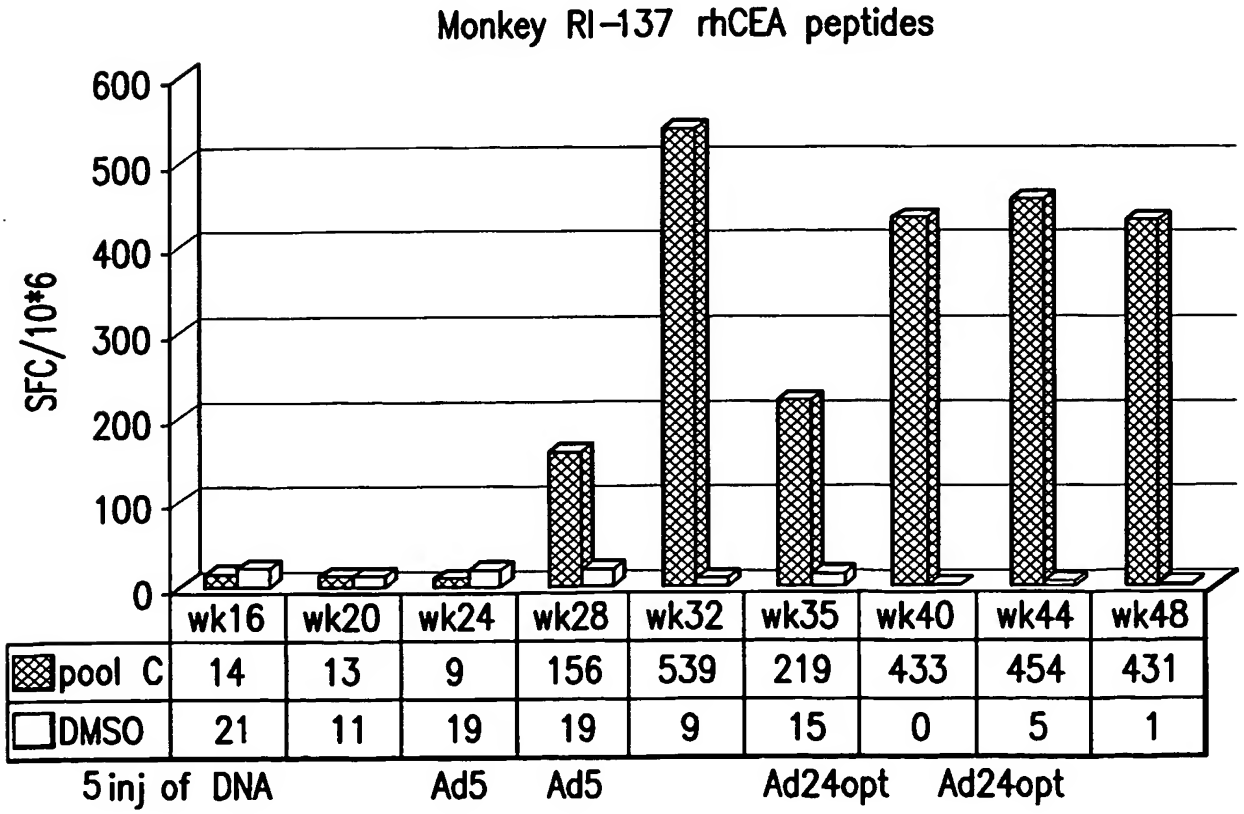

FIG.12B

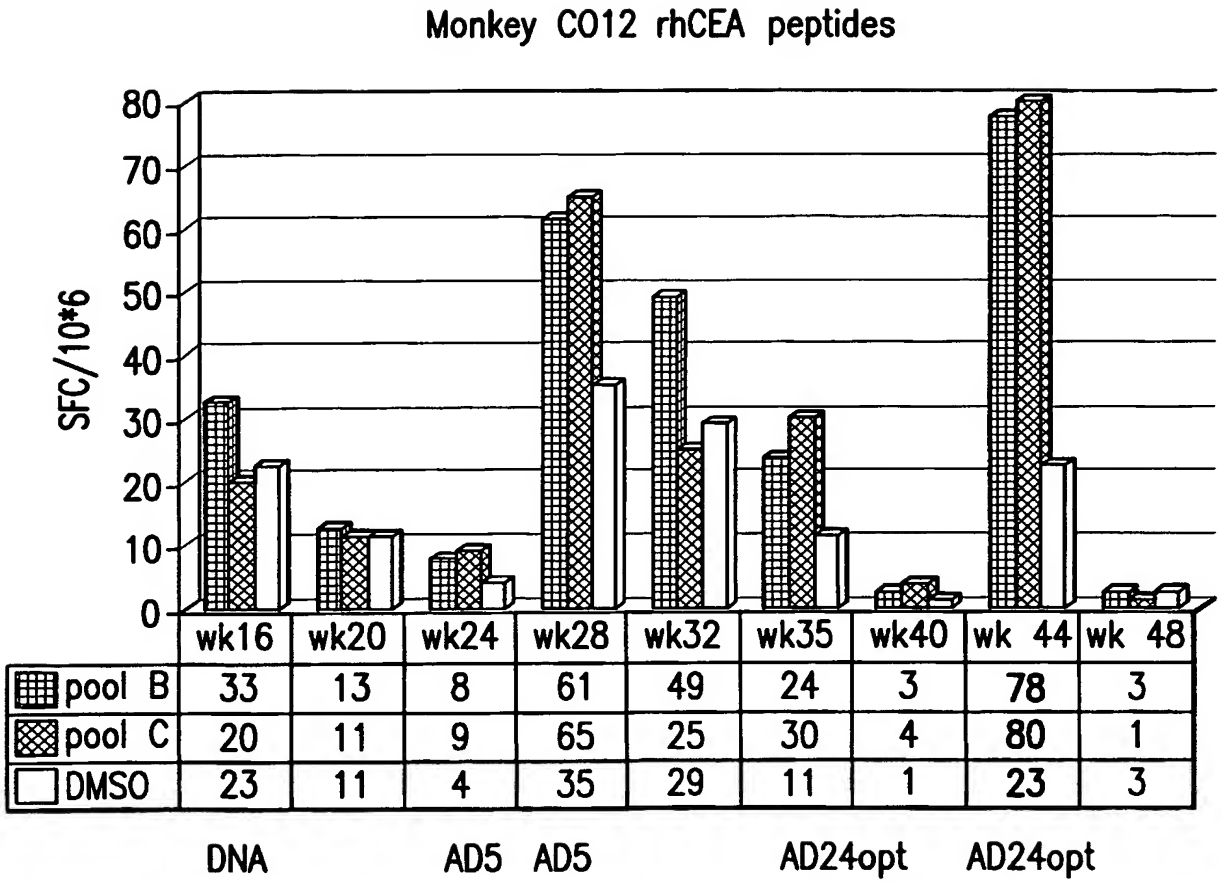
Monkey CO12 rhCEA peptides
SFC/10*6
80
70
60
50
40
30
20
10
0
wk16 wk20 wk24 wk28 wk32 wk35 wk40 wk 44 wk 48
pool B 33 13 8 61 49 24 3 78 3
pool C 20 11 9 65 25 30 4 80 1
DMSO 23 11 4 35 29 11 1 23 3
DNA
AD5 AD5
AD24opt AD24opt

FIG.12C

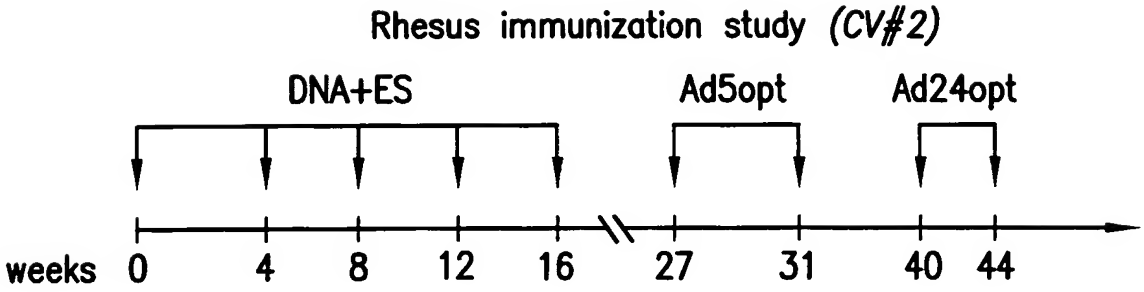
Rhesus immunization study (CV#2)
DNA+ES
Ad5opt
Ad24opt
weeks
0
4
8
12
16
27
31
40
44

FIG.13A

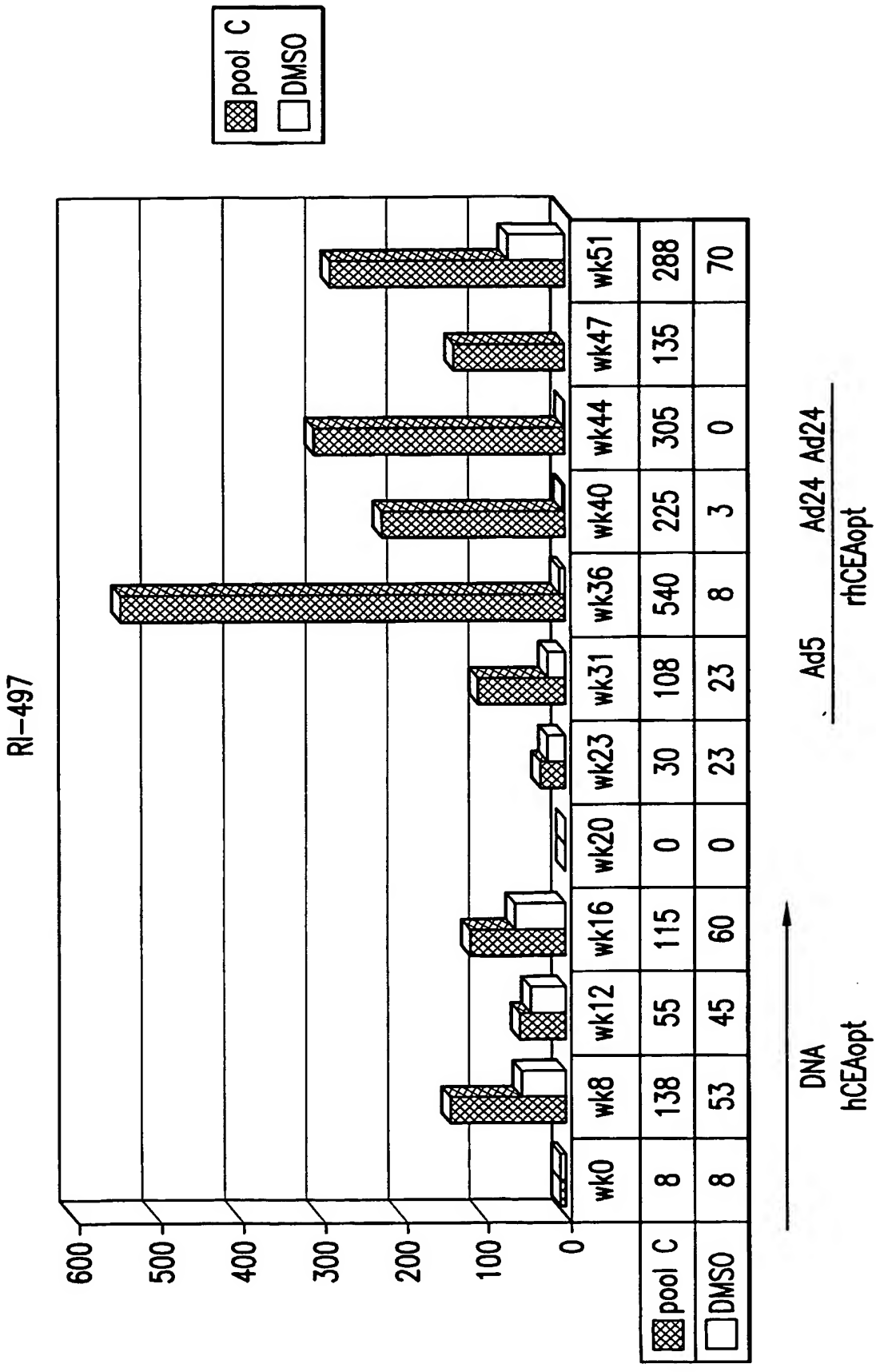
pool C
DMSO
RI-497
600
500
400
300
200
100
0
wk0 wk8 wk12 wk16 wk20 wk23 wk31 wk36 wk40 wk44 wk47 wk51
pool C 8 138 55 115 0 30 108 540 225 305 135 288
DMSO 8 53 45 60 0 23 23 8 3 0 70
DNA
hCEAopt
Ad5 Ad24 Ad24
rhCEAopt

FIG.13B

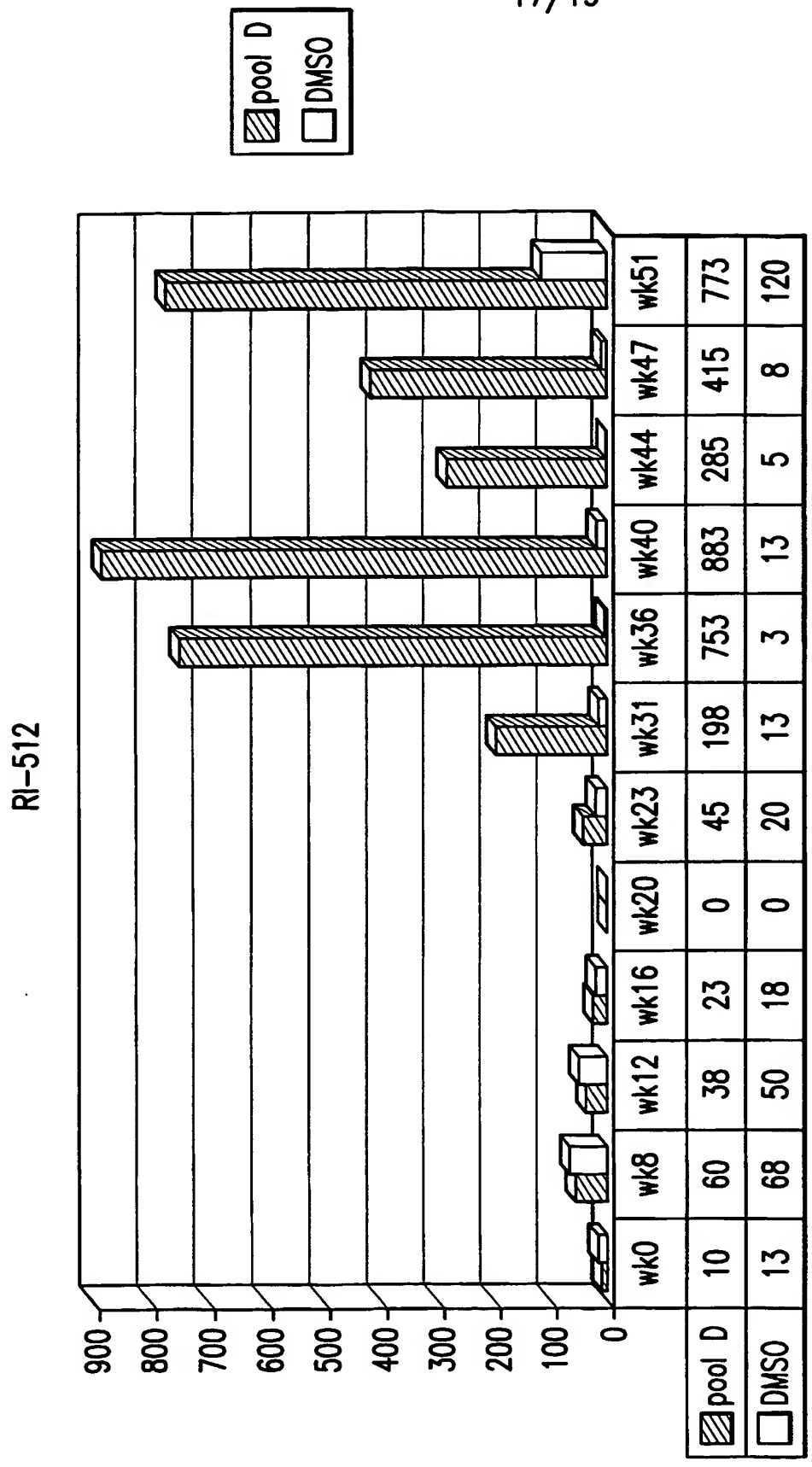
pool D
DMSO
RI-512
900
800
700
600
500
400
300
200
100
0
wk0 wk8 wk12 wk16 wk20 wk23 wk31 wk36 wk40 wk44 wk47 wk51
pool D 10 60 38 23 0 45 198 753 883 285 415 773
DMSO 13 68 50 18 0 20 13 3 13 5 8 120
DNA
hCEAopt
Ad5 Ad24 Ad24
rhCEAopt

FIG.13C

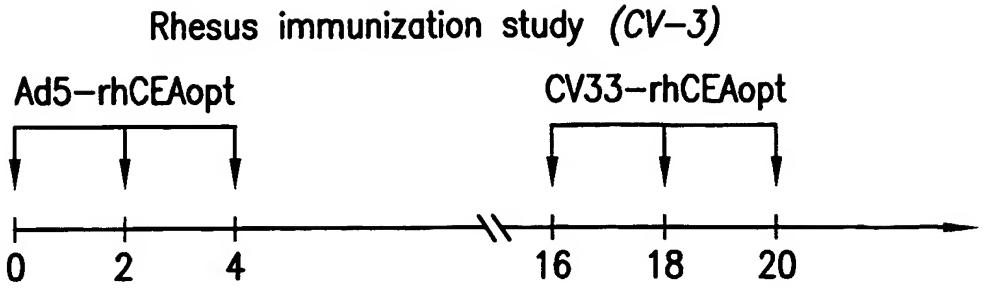

FIG.14A

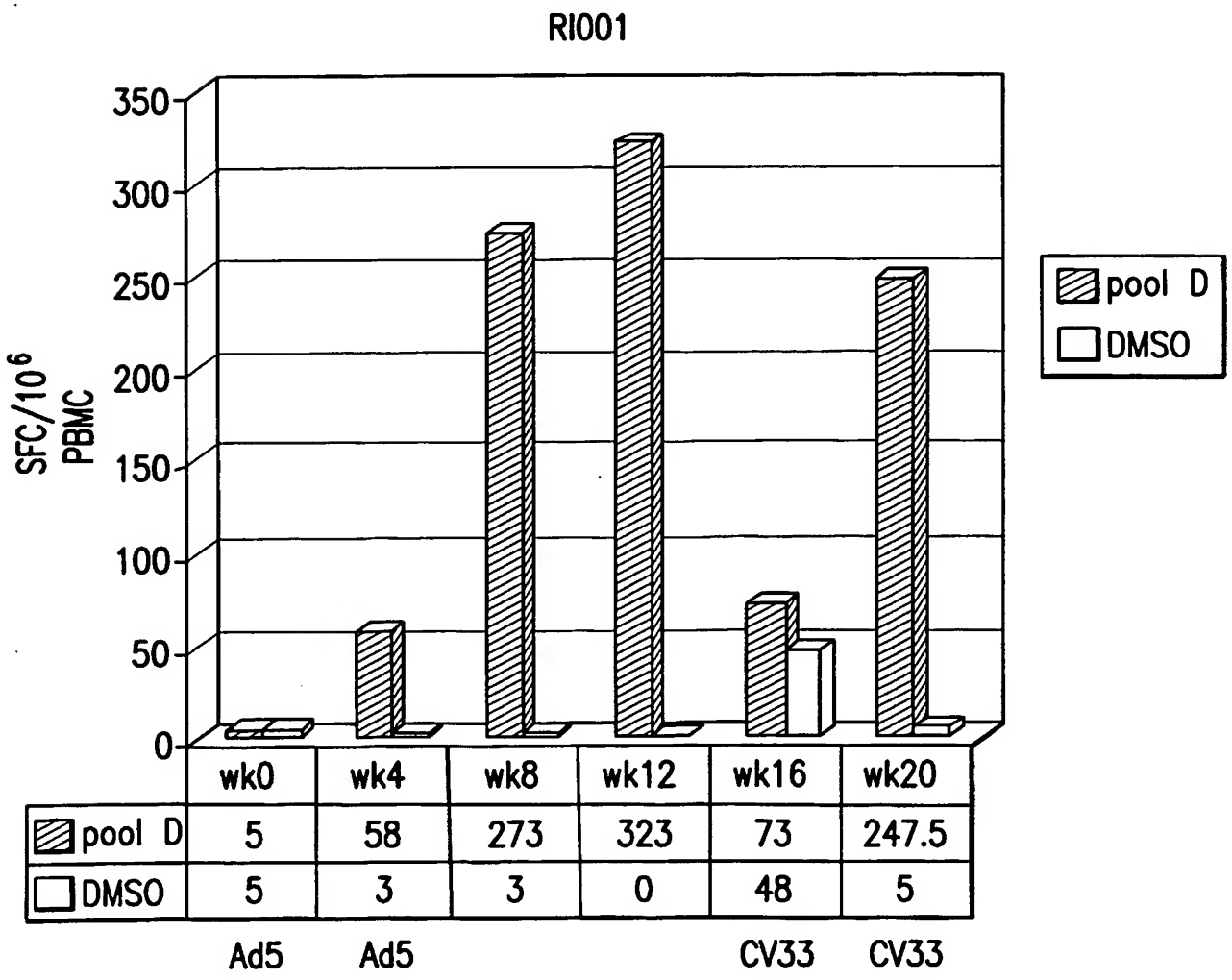

FIG.14B

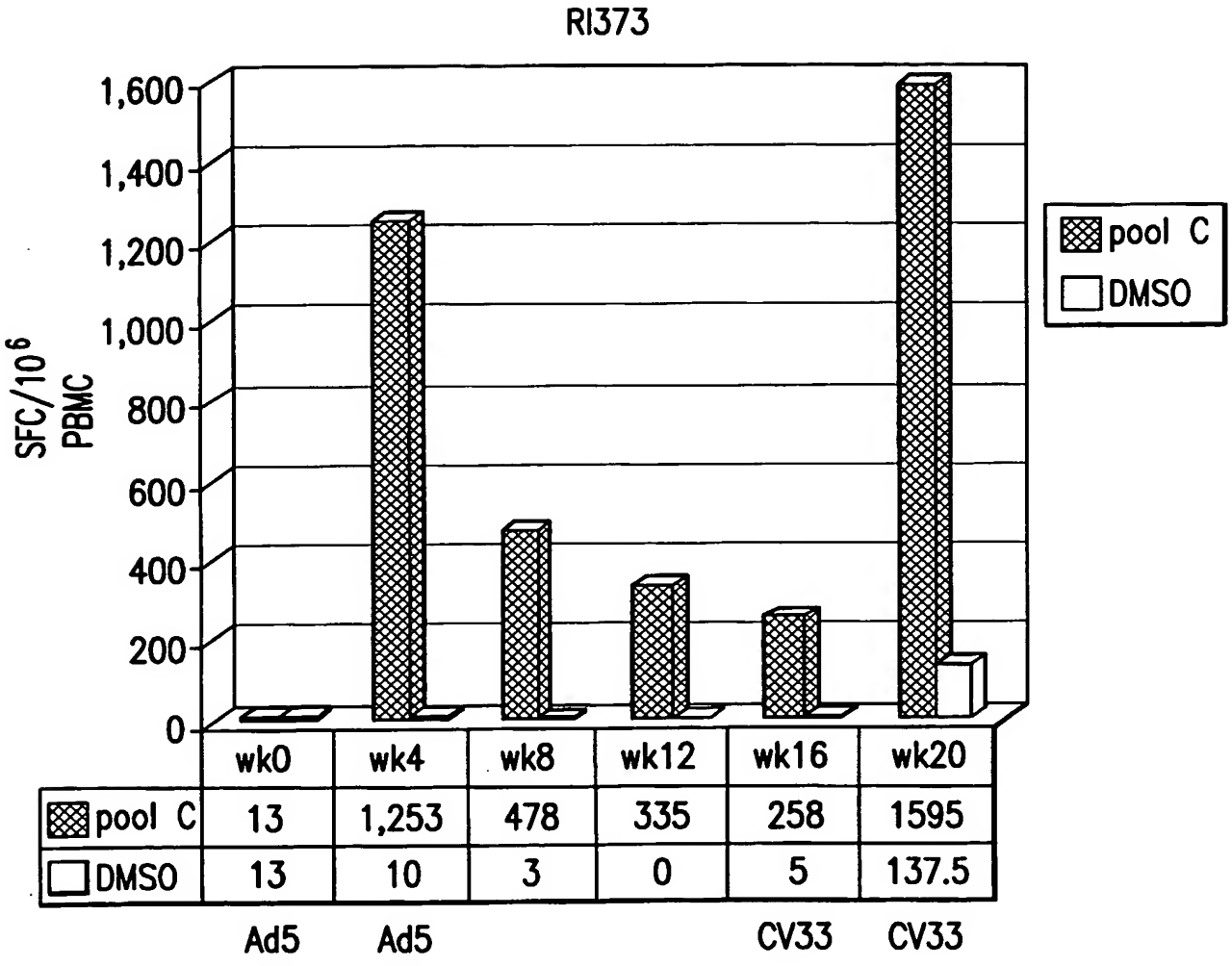
RI373
SFC/10^6 PBMC
1,600
1,400
1,200
1,000
800
600
400
200
0
pool C
DMSO
wk0 wk4 wk8 wk12 wk16 wk20
pool C 13 1,253 478 335 258 1595
DMSO 13 10 3 0 5 137.5
Ad5 Ad5 CV33 CV33

FIG.14C